Something old, something new: strategies for controlling pernicious weeds and restoring habitat of conservation value using *Pteridium aquilinum* as a test case

G MILLIGAN, E S COX, J G ALDAY, V M SANTANA, |H A MCALLISTER,[*]R J PAKEMAN, M G LE DUC & R H MARRS

School of Environmental Sciences, University of Liverpool, Liverpool, L69 3BX, UK

[*]*The James Hutton Institute, Craigiebuckler, Aberdeen AB15 8QH, UK*

Milligan, G. greggmilligan@icloud.com

Cox, E.S. emma@northpenninesaonb.org.uk

Alday, J.G. josucham@gmail.com

Santana, V.M. vm.santana@ua.es

McAllister, H.A. sorbus@liv.ac.uk

Pakeman, R.J. Robin.Pakeman@hutton.ac.uk;

Le Duc, M.G. mled@liv.ac.uk

Corresponding author: Marrs, R.H calluna@liv.ac.uk

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13/7/2015

Summary

There is a need for management strategies to control dominant perennial weeds and restore semi-natural communities. We compared the effects of five weed-control treatments on dense *Pteridium aquilinum* (L. Kuhn) relative to an untreated experimental-control over an eight-year period with the aim of restoring acid-grassland. The weed control-treatments tested were: cutting and bruising, both twice and thrice annually, and herbicide treatment (asulam in year 1 followed by annual spot-re-treatment of all emergent fronds). *P. aquilinum* performance and plant species composition were monitored. Data were analyzed using Bayesian mixed-effect models and multivariate techniques. Cutting twice and thrice yearly and the asulam treatment all reduced frond density to zero, both bruising treatments were ineffective. The plant communities in the cut and asulam-treated plots showed differences from the untreated and bruised plots: the asulam-treated plots contained more ruderal species and the cut plots were more typical of acid-grassland. Acid-grassland recovery was fastest in the asulam-treated plots, but the cut plots caught up after approximately five years. There were two important conclusions. First, an intractable weed like *P. aquilinum* can be eradicated and a vegetation more suited for grazing can be achieved by the continuous application of some treatments over many years. Here success was achieved by cutting twice/thrice annually, or by a single asulam application followed by annual spot-spraying of all emergent fronds for eight years Second, bruising, a treatment favoured by some conservation organizations, did not work and cannot be recommended. The potential for the use of long-term, continuously-applied treatments might be considered for all perennial weeds with large underground root\rhizome systems.

Keywords: Bayesian Mixed-effects Models, Canonical Correspondence Analysis, *Pteridium aquilinum*, asulam, cutting, bruising.

Introduction

Perennial weeds with an extensive underground root/rhizome system cause major problems for agriculture, especially in extensively-grazed systems, and plant community conservation worldwide

(Naylor, 2002). Once established, they can come to dominate the ecosystem, becoming an effective mono-culture with few other species present. Attempts to restore such invaded ecosystems must involve at least two inter-linked processes. First, the weed species must be either eradicated or be reduced below the level at which it loses its competitive advantage. Second an appropriate, and stable, native community must be established (Alday *et al.*, 2013). Where such weeds have a large store of underground reserves, control can be very difficult; there are many examples where after good initial control there is a rapid return of the weed species (Lowday & Marrs, 1992). Clearly, the only way to test appropriate management for both weed control and restoration of appropriate plant communities is through experiments where management interventions are followed over many years. Here, we assess weed control and concurrent restoration over eight years using *P. aquilinum* (L.) Kuhn as the test weed species.

Pteridium is a world-wide perennial-weed problem (Marrs & Watt 2006; Miatto *et al.*, 2011), capable of producing a dense canopy and often dominating the invaded communities, reducing economic and conservation value, and the aesthetic appeal of natural areas (Pakeman & Marrs, 1992; Marrs & Watt, 2006). In Britain, the current ecological success of *P. aquilinum* has been suggested to result from changes in cultural and environmental practices which have increased the amount of suitable habitat, and this, combined with improved climatic conditions, has provided opportunities for range expansion (Pakeman *et al.*, 2000). *P. aquilinum* possesses several biological and ecological attributes that allow it to capitalize on these opportunities for range expansion: (i) an extensive rhizome system possessing considerable stores of carbohydrate, nutrients and dormant buds, (ii) high productivity, capable producing a dense canopy that casts deep shade over understory vegetation, (iii) deep litter accumulation which suppresses colonization of other species, and (iv) possibly production of allelopathic substances (Marrs & Watt 2006).

The attributes which contribute to the ecological success of *P. aquilinum* also provide it with a resilience to control treatment, which is often deemed difficult (Lowday & Marrs, 1992; Chapman *et al.*, 2009). Over the last 40 years, control has usually been implemented either by cutting or herbicide use (usually asulam). Cutting aims to continuously deplete rhizome carbohydrate and nutrient stores (Williams & Foley, 1976; Marrs & Watt, 2006) resulting in a slow reduction in frond cover to a very low level (Lowday & Marrs, 1992; Marrs *et al.,* 1998); in the longest experiment carried out to date a few fronds remained even after 18 years of twice-yearly cutting (Marrs *et al.,* 1998). Cutting also breaks up the litter layer accelerating vegetation recolonization (Marrs *et al.,* 2007).

Asulam is a pteridophyte- and *Rumex*-selective post-emergence, systemic-carbamate herbicide (Lowday & Marrs, 1992), considered to be of relatively low ecological risk (USEPA, 1995) and it has been licensed for aerial application in the UK. In 2012, asulam was withdrawn from use within the European Union (EU) because of a lack of information with respect to metabolites resulting from its breakdown in crop plants (European Union Regulation (EC) No. 1045/2011 (Anon 2011). However, its use has been continued as a result of derogated powers in four EU-member states, including the UK, and asulam is currently going through the EU re-registration/approval process.

Recently, however, two alternative approaches have been suggested for *P. aquilinum* control within the UK. The first is asulam application to provide an initial reduction in *P. aquilinum* cover followed by spot-spraying all emergent fronds in subsequent years, without respite. Indeed, this has been suggested as an effective way of eradicating *P. aquilinum* (Robinson, 2000). The second is the re-introduction of bruising (variously termed breaking, crushing and rolling) as an alternative to cutting. Bruising aims to knock the fronds down and produce breaks/nicks along the frond rachis, which is damaged, but not severed (Braid, 1959). This approach was commonly used in Britain up to the Second World War before the advent of adequate cutting machines and effective herbicides (Braid, 1959). Bruising is currently undergoing a revival of interest for a variety of reasons; it can be incorporated into organic farming regimes where it is preferred over herbicide use, and can be applied much faster than cutting especially on rocky, steep or uneven ground, (Bacon *et al.,* 2001; Lewis *et al.,* 1997). Neither bruising nor continuous spot-spraying has been assessed experimentally in randomized-control trials.

Here, therefore, we compared the effectiveness of bruising (twice- and thrice-yearly) and annual spot-spraying of asulam against two cutting treatments (twice- and thrice-yearly) and an untreated “experimental-control”. We tested three hypotheses:

(1) Can intensive treatment eradicate *P. aquilinum* through either ;

  a. multiple annual cuts/bruisings, or

  b. an initial asulam application plus annual follow-up spot-spraying?

(2) Is bruising an effective means of *P. aquilinum* control; and if not suggest potential reasons?

(3) Do the differing treatments produce different trajectories of change in the underlying plant community?

Methods

Experimental Design

The experiment was set up at Bamford Edge in the Peak District National Park, Derbyshire, UK (1^{o}41' W 53^{o}41' N; GB National Grid Reference SK213 841). The site is a steep escarpment, most of which has been covered with *P. aquilinum* for over a century (N Taylor, pers. comm.). The site was sprayed with asulam in 1990 (Marrs *et al.,* 1992), but by 2005 there had been substantial frond recovery. The soil is a podzol with a pronounced organic layer and the site is grazed by sheep at ca. 0.5 sheep ha^{-1} in keeping with agri-environment scheme prescriptions (Alday *et al.,* 20013).

In November 2004, three replicate-blocks, each containing six 20 m x 20 m plots separated by 2 m strips, were marked out; this plot size exceeds that need to remove violations of independence stemming from between-plot interference from underground rhizome effects (Le Duc *et al.,* 2003). Plots were selected randomly for application of one of six treatments: (i) *P. aquilinum* cut-twice yearly (Cut 2x); (ii) *P. aquilinum* cut-thrice yearly (Cut 3x); (iii) *P. aquilinum* bruised-twice yearly (Bruise 2x); (iv) *P. aquilinum* bruised-thrice yearly (Bruise 3x); (v) overall asulam application in 2005 followed by annual spot-spraying with asulam of all emerging fronds (Asulam), and (vi) the untreated "experimental control" (Untr). The experimental layout is illustrated in Fig.S1 (Supporting information). The strips around, and within, each block were bruised thrice per year for access.

Treatments

Cutting and bruising were both applied in late-June (x2, x3), late-July (x2, x3) and late-August (x3) from 2005 to 2012 inclusive. Peak frond biomass usually occurs in late-July (Williams & Foley, 1976) and the timing was recommended by Braid (1959) for cutting, i.e. to produce three flushes of fronds to be treated each season. In the cut treatments the fronds were cut using a petrol-driven strimmer; bruising was carried out using a "bracken-bruiser" (supplied by Peter Gotham, Bracken Bruisers, Sidmouth, UK), trailed by a 4WD ATV (Fig. S2, Supporting information). Over the eight years of the study, cutting/bruising was applied 16 times to the x2 plots and 24 times to the x3 plots. The herbicide treatment followed methodology proposed by Robinson (2000) for *P. aquilinum* clearance and started with an initial application of asulam (commercial product, Asulox®, produced by Bayer CropScience Ltd and United Phosphorus Ltd) by knapsack sprayer at a rate of 4.4 kg asulam ha^{-1} (11 litres Asulox ha^{-1}) in 400 ml water in early September 2005. Thereafter, follow-up spot-spraying of every emergent frond was carried out annually from 2006 to 2012 (7 spot-applications) using a knapsack sprayer at a dose of approximately 2 ml per squirt at a ratio of 6% vol:vol Asulox to water (~0.05g asulam per frond). From 2009-2012, the number of spot-spray-squirts were counted in each plot to provide a whole-plot estimate of success.

Monitoring

Each treatment plot was divided into a grid of 1 m^2 squares. Every June, from 2005 (pre-treatment) to 2013 inclusive; a 1 m^2 quadrat was placed at five different randomly-selected co-ordinates each year within this grid. The cover (%) of all plant species present in each quadrat was estimated visually; species nomenclature follows Atherton *et al.* (2010) for bryophytes and Stace (2010) for vascular plants. A 0.25m^2 sub-quadrat was then placed centrally and all *P. aquilinum* fronds were cut at soil level, counted to provide an estimate of density (corrected to number m^{-2}) and length measured to provide a measure of *P. aquilinum* productivity (mean length per quadrat was calculated). The results for the 2013 sampling reflect the response to the last treatment application in 2012.

By 2013, it was apparent that the asulam and both cutting treatments reduced the *P. aquilinum* infestation to a very low level. Accordingly in September 2013, an additional whole-plot sampling of these treatments was performed; here the replicate plots were gridded into 100 2m x 2m cells and the fronds in each counted.

Data Analysis

All data analyses were performed using R version 2.13.2 (R Development Core Team, 2011).

Five response variables, *P. aquilinum* cover, frond density (frond m^{-2}), mean frond length (cm), species richness and Shannon-Weiner species diversity (*H*) were analyzed to assess treatment effects through time on *P. aquilinum* performance and plant community diversity. When assessing *P. aquilinum* performance in control experiments it is important to consider both frond length and density because *P. aquilinum* often exhibits a trade-off between them especially in cutting treatments (Lowday et al. 1992); cover was also included because this is the easiest one to assess under field conditions by managers. Given the randomized-block design of this experiment an error term was needed to account for any block and/or plot-level variation. Moreover, initial analyses of the temporal effects for each treatment identified heterogeneous variance structures. Therefore, it was deemed more suitable to analyze these data using Generalized Linear Mixed Models (GLMMs) in a Bayesian framework (as opposed to the somewhat simpler frequentist ANOVA/ANCOVA approach) as this offered a more robust method for estimation of the parameters and their associated confidence intervals, whilst also circumventing problems associated with data transformation (Pinheiro & Bates, 2000; Bolker *et al.*, 2009). Hence, here all data were analyzed untransformed. All univariate analyses were implemented using the 'MCMCglmm' function in the 'MCMCglmm v.2.16' package (Hadfield, 2010); models incorporated treatment and a treatment x time interaction as fixed terms and block and plot identity as random covariates; Polynomial contrasts were included (Gurevitch & Chester 1986), but only the first-order ones are discussed here. A Markov Chain Monte Carlo (MCMC) routine was used to estimate the posterior distributions for the mean effects and their corresponding 95% confidence intervals and associated Bayesian *p*-values (P_{MCMC}). The models incorporated parameter-expanded priors. All models were run for a 1 x 10^5-iteration burn-in with sampling every 500th iteration for a further 2 x 10^6 iterations, resulting in an effective sample for each parameter estimate of 4 x 10^3 from the approximated posterior distribution. Trace plots of all parameters were checked for convergence.

In addition, frond distribution maps for the 2013 whole-plot assessment were created using the 'interp', 'image' and 'contour' functions within the 'akima' package v.0.5-11 (Crawley, 2007).

Species composition data (Hellinger-transformed, function 'decostand') with respect to each treatment through time was analyzed using Canonical Correspondence Analysis (CCA, function 'cca') in the 'vegan' package v.2.0-2 (Oksanen *et al.*, 2011). Hellinger transformation does not weight rare species differentially and has been shown to be appropriate for testing the significance of relationships between community composition and a set of explanatory variables, particularly when there are many zero values within the data (Legendre & Gallagher 2001). Significance of the overall model, the first canonical axis and treatment effects were tested using Monte Carlo tests with 999 permutations.

Results

Treatment effects on P. aquilinum *and species diversity*

All first-order GLMM-estimated results for the four response variables between 2005 and 2013) are presented in Table 1 along with assessment of significant treatment effects relative to the untreated response. At the start of the experiment in 2005, there were no significant differences ($P<0.05$) between treatments in terms of frond length (overall experimental mean±SE, n=18; 35.9±3.6cm), frond density (30.8±3.6 fronds m^{-2}), cover (23.0±2.8%), rhizome mass (2.11±0.75 kg m^{-2}), *P. aquilinum* litter cover (49.4±4.6%), cover of understorey species (5.08±4.3%) or Shannon-Weiner Index (1.38±0.06; all mean±SE, n=18). Thereafter, *P. aquilinum* cover showed significant responses through time for all treatments ($P<0.001$): in three treatments there was an increase between 2005-13; the untreated controls showed the largest increase (53%) closely followed by thrice- and twice-yearly bruising with a 50% and 35% increase respectively (Fig. 1a). The remaining three treatments showed a reduction in cover; 16% in cutting thrice-yearly, 17% in the asulam treatment and 19% in cutting twice-yearly (Table 1, Fig. 1a).

No significant effect through time was found for frond density in the untreated plots ($P >0.05$, Table 1. Fig. 1b), but an increased frond density of +15 fronds m^{-2} ($P<0.05$) and +35 fronds m^{-2} ($P<0.001$) over the starting density of 31 fronds m^{-2} in the twice- and thrice-bruised plots (Fig. 1b) respectively. The bruising treatments showed a dip in frond density between 20007-11 but then there was strong increase (Fig.1b). The cutting and asulam treatments reduced frond density over the starting density; -15 fronds m^{-2} in the asulam treatment ($P< 0.05$), -19 fronds m^{-2} in the thrice-yearly cut ($P<-.0.05$) and -24 fronds m^{-2} in the twice-yearly cut ($P<0.001$) Fig. 1b). The reduction in frond density through time in the asulam treatment was reflected at the whole plot-scale with respect to the number of fronds squirted each year during spot-spraying (Fig. 2).

The only significant increase in mean frond length was found in the untreated plots (increase of 32 cm, P<0.001). A reduction of 27.1 cm was found in the asulam-treatment and 16.1 cm and 22.0 cm in the twice and thrice-yearly cut respectively (all P<0.001). No significant effects through time were found for either of the two bruising regimes on frond height (Fig. 1c).

No significant treatment effects were found for species richness but significant increases in Shannon-Weiner index (H) was found for two treatments, cutting twice and thrice yearly where the index increased to 0.34 and 0.31 respectively (both (P<0.001, Fig. 1d).

The spatial distribution of all three replicate blocks for the three treatments that reduced the *P. aquilinum* infestation successfully (Fig. 3) showed that frond density in the middle of each plot had been reduced to effectively zero. Almost all fronds were located around the periphery and the densities around the periphery were greatest in the cut treatments.

Treatment effects on plant community composition

The CCA explained approximately 11.0% of the species-treatment variance with eigenvalues for the first two axes of 0.16 and 0.08 respectively. Both the overall model and first canonical axis were significant (P = 0.005). With the exception of twice-yearly bruising (P = 0.10) all other *P. aquilinum*-control treatments had significant overall effects on community composition (P <0.01), and significant interactions through time (P <0.01). The species biplot showed most of the dominant species, typical of acid-grassland close to the centroid (*P. aquilinum, Agrostis capillaris, A. vinealis, Deschampsia flexuosa)* with the less common species around the edges (Fig. 4a). The untreated plots showed a trajectory increasing in the direction of *P. aquilinum* and away from grassland species (Fig. 4b). Both bruising treatments showed a less well pronounced trajectory in approximately the same direction. The asulam spot-sprayed plots showed an opposite response moving away from *P. aquilinum* and towards a community dominated by ruderal species (*Aira praecox*, *Epilobium angustifolium*, *Poa annua*) whereas both cutting treatments showed an almost orthogonal response to the untreated/bruising-asulam trajectories with a positive correlation with acid-grassland species (*Cerastium fontanum*, *Galium saxatile*, *Rumex acetosella* and *Stellaria media*) and a negative one with a group of species in the lower quadrant of Fig 4; these were a *Carex* spp., a *Fraxinus excelsior* seedling, *Luzula multiflora*, *Veronica officinalis* and the bryophytes *Hypnum cupressiforme*; *Lophocolea bidentata* and *Ptilidium ciliare*.

Discussion

Can intensive treatment eradicate P. aquilinum *(Hypothesis 1)?*

To test this hypothesis it is necessary to consider the results obtained here with those of a multi-site study testing some of these treatments in different regions of Great Britain (Alday *et al.*, 2013). The Bamford Edge site would be expected to develop into an acid-grassland and Alday *et al.* (2013) suggested that such sites would produce alternative stable acid-grassland states with either annual cutting once or twice yearly, or with a single asulam application for at least 10-years. However, it has also been established that *P. aquilinum* recovers from single initial asulam applications (Robinson, 1986; Lowday & Marrs, 1992). The Bamford site used here was treated with asulam by helicopter in 1990 (Marrs *et al.*, 1992) and two of the results from the untreated plots reported here suggest that they conform to the generalizations of Alday et al. (2013). At the start of the present study, 15 years after the 1990 asulam application, the *P. aquilinum* cover was relatively low (~50%) but increased continuously to ~ 100% by 2013, and there was at least some species in the understory (cover 5%) and diversity did not show a significant decline through time. These results suggest that the alternative stable state described as possible for at least a 10-year period under experimental conditions (Alday *et al.*, 2013) might be happening here, but that it has started to break down after about 15-20 years after the P. *aquilinum* was sprayed. This suggests that the understory species are relatively resilient in that they maintain low cover values for at least 23 years. This suggests that the understory flora can persist for a long time at low cover and provide a reservoir for revegetation if the *P. aquilinum* cover is reduced in future control programs. These conclusions are speculative and it is possible that the retention of the understory species in our experimental plots is a function of elevated side-light and other disturbance associated with the management of these experimental plots. Nevertheless, our hypotheses must be tested on the basis that the *P. aquilinum* infestation is increasing in the untreated controls and the underlying species diversity is low, but stable.

Here, we demonstrated that three *P. aquilinum* control treatments were extremely effective (asulam once followed by annual spot spraying of individual fronds, and cutting twice or thrice yearly) producing a complete frond clearance within eight years from the majority of the experimental plot area; remaining fronds being restricted to the periphery of the treated areas, growing in from the plot edges (Hypothesis 1 accepted for these three treatments). The two bruising treatments were ineffective (Hypothesis 1 rejected, see Hypothesis 2 below). Here, the asulam treatment produced the fastest impact on *P. aquilinum*. A single asulam application is usually very effective in reducing initial *P. aquilinum* frond density and cover, but there is often a relatively rapid recovery within 5-10 years (Lowday & Marrs, 1992; Marrs *et al.*, 1998). In previous long-term experimental studies of *P. aquilinum* control where the cut twice yearly option and single asulam treatments have been tested, complete eradication has not been achieved in 10 years (Alday *et al.* 2013) and 18 years (Marrs *et al.*, 1998); in these two studies the *P. aquilinum* frond reduction was to a very low level but it was never zero. From a land-management perspective, Alday *et al.,* (2013) suggested that mechanical methods were preferred on sites where there was deep litter layer and a depauperate understory, but herbicide use produced faster results and was less expensive to implement where a diverse grassland understory community was present.

It is worth speculating on the possible cause of this success on the Bamford site. One reason might be differential sheep-grazing pressure. Whilst the sheep grazing pressure was at the ESA prescription level of 0.5 ha^{-1}, but it was obvious that the sheep spent a great deal of time in the cut and asulam-treated plots) and grazed the vegetation to a relatively low height (< 5cm, Figs S1, S3, Supporting information). It is also very difficult to prove complete eradication especially for weedy species like *P. aquilinum* that persist and reproduce mainly though rhizomes. Rhizomes can remain dormant for up to 10 years (Marrs & Watt, 2006) but there is no information on the length of time that rhizomes persist when they have been subject to impacts of considerable control treatment. Nevertheless, true eradication cannot be proven until a relatively long "*P. aquilinum*-free" period has been demonstrated. An assessment of future *P. aquilinum* recolonization of these treated plots at Bamford Edge might help in separating the relative role of edge invasion versus recovery from extant dormant rhizomes. The plot separators in this study were bruised thrice annually and responded in the same way as the bruised treatment plots, i.e. poor *P. aquilinum* control. Edge invasion was detected in both cutting treatments from *P. aquilinum* in the bruised pathways, but this was absent from the asulam plots (Fig.3) and this suggests that where cutting is used to treat a sub-area of *P. aquilinum*, continued asulam treatment along the border will help keep it in check and prevent re-invasion. This requires further test.

What these results also demonstrate is that to get very good *P. aquilinum* control, a long-term strategy needs to be adopted and here an eight year program was needed; in the cut plots 16 and 24 cuts were applied in the twice and thrice yearly treatments, and in the asulam treatment there was the initial overspray plus seven annual spot-sprays. Thus to achieve these good results requires intensive management, which is both expensive in time and resources (cutters, fuel, labour, herbicides).

The effectiveness of the asulam treatment plus follow-up spot-spraying is further evidence of its value in *P. aquilinum* control as it is the only effective, selective herbicide available which can be applied from the air over rough terrain at the present time (Robinson 1986, 2000). Application techniques have been developed specifically to spot-spray *P. aquilinum* on rough terrain but they require a relatively large number of operatives (Robinson 2000; Fig. S4, Supporting information). Moreover, with spot-spraying the reduction in number of squirts required per plot follow the law of diminishing returns; whilst it is easy to enforce this treatment under experimental conditions, it will be harder to maintain enthusiasm in practical land management.

Is bruising an effective means of P. aquilinum *control (Hypothesis 2)?*

Bruising is a relatively old technique that was commonly used before the development of suitable cutting equipment and selective herbicides. As rugged cutters and herbicides became available bruising fell out of favour, but is now seeing a resurgence in popularity especially for conservation use (Bacon *et al.,* 2001; Lewis *et al.,* 1997). In the bruised plots, *P. aquilinum* cover and frond density increased through time and there was no reduction in frond length. Indeed, the bruised plots resembled the untreated experimental controls (Hypothesis 2 rejected, Fig. S3, Supporting information). Nevertheless, there are accounts of bruising providing better results than obtained here (Braid, 1959; Lewis *et al*., 1997), and it is difficult to reconcile this.

There are, however, some advantages to bruising over cutting in bruisers are more robust and can be towed faster speeds than cutters, and hence more land can be covered in the same time. Moreover, they can be towed by heavy horse and this allows access to some land that is inaccessible even to ATV vehicles (Fig. S4, Supporting Materials). Nevertheless, experience here shows that it is not possible to guarantee good *P. aquilinum* control even with double- and triple-passes each year over an eight-year period. A further drawback to bruising is that the bruising bars/flanges can (like cutters) damage and uproot stones (R.H. Marrs pers. comm.). This is not necessarily a problem but clearly both are inappropriate where there is archaeological interest (Pakeman *et al.*, 2007), and an herbicidal approach preferred. The variability in efficacy of bruising was noted by Braid (1959) who stated "recently the maker of a well-known bracken bruiser confessed his preference for cutting (Henderson, 1954)".

One reason for the poor performance reported here is almost certainly the lack of damage to the vascular strands during bruising (Fig. 5) with physiological processes (photosynthesis, transpiration) remaining at similar rates above the bruise to untreated stands for several weeks (Cox, 2007). We can, therefore, assume that carbohydrate and hormone flows to the rhizome continue, where the latter should enforce bud dormancy and prevent the new waves of frond emergence so obvious after cutting (Lowday *et al.*, 1983). This reduced frond production will, therefore, result in much less removal of rhizome resources. These preliminary speculations require confirmation using tracers to study carbohydrate/nutrient fluxes between fronds and in intact and bruised *P. aquilinum*.

Do the differing treatments produce different trajectories the underlying plant community (Hypothesis 3)?

The species detected here are representative of many depauperate, calcifuge vegetation communities that are widely-distributed in the UK uplands at altitudes below 600 m (Rodwell, 1992). The treatments applied here did not affect species richness, but the Shannon-Weiner diversity index was increased only by cutting twice and thrice yearly. Both cutting treatments and the asulam one produced a grassland community containing at least some species important for sheep grazing, especially the grasses *A. capillaris* and *F. ovina* (Rawes & Welsh, 1969). The multivariate analyses, however, indicated divergence in community composition within the sub-ordinate species through time between the treatments, with the untreated control and both bruising treatments moving towards greater *P. aquilinum cover* with a lower abundance of all other species. The asulam and cutting treatments were most effective in that they both retained a greater species complement and showed an increasing divergence from both untreated plots and bruising treatments. Nevertheless, whilst the asulam and cutting treatments had many species in common, the asulam treatment produced a sward with a greater component of annuals and ruderals (*Aira praecox*, *Poa annua*, *Epilobium angustifolium*) with a relatively low grazing value, whereas the cutting treatments had a greater cover of typical upland, acid-grassland species which will enhance the grassland quality for sheep grazing (Rawes & Welch, 1969).

Conclusions

This study has shown that under some circumstances it is possible to clear *P. aquilinum* from heavily-infested land using either cutting or herbicide, but it required a considerable time (8-years here), and substantive investment. However, a different vegetation trajectory resulted depending on the control-strategy adopted. Nevertheless, we showed that sustained weed control over a considerable time period using either cutting or repeated applications of an appropriate selective herbicide was extremely effecting for *P. aquilinum*. It is possible that a similar strategy would work for controlling other intractable perennial weeds worldwide, especially those with large underground resources Bruising was ineffective here.

Acknowledgements

This experiment was set up under a DEFRA-funded contract (BD1226) and continuation has been supported by both the Heather Trust and the Ecological Continuity Trust. We thank J. Archdale and N. Taylor for site access and logistic support, and M. Harris, M. O’Connor, J. Ghorbani, K. Hatton and T. Tollitt for technical support. Peter Gotham provided the bracken bruiser and asulam was provided by Bayer CropScience Ltd and United Phosphorus Ltd.

References

ANON (2011) Commission implementing regulation (EU) no 1045/2011 of 19 October 2011. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:275:0023:0024:EN:PDF. [accessed 18/11/2013].

ALDAY JG, COX ES, PAKEMAN RJ, HARRIS MPK, LE DUC MG & MARRS RH (2013) Overcoming resistance and resilience of an invaded community is necessary for effective restoration: a multi-site bracken-control study. *Journal of Applied Ecology,* **50**, 156-167.

ATHERTON I, BOSANQUET S & LAWLEY M (2010) *Mosses and Liverworts of Britain and Ireland - a field guide*. British Bryological Society, Plymouth, UK.

BACON, J BARNES, N, COLESHAW, T, ROBINSON, T & TITHER, J (2001) *Practical solutions. Equipment, techniques and ideas for wildlife management*. Forum for the Application of Conservation Techniques, English Nature, Peterborough, UK.

BOLKER BM, BROOKS ME, CLARK CJ, GEANGE SW, POULSEN JR, STEVENS MHH & WHITE JSS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution*, **24,** 127–135.

BRAID KW (1959) Bracken: a review of the literature. CAB, Hurley. UK.

CHAPMAN DS, TERMANSEN M, QUINN CH, JIN N, BONN A, CORNELL SJ, FRASER EDG, HUBACEK K, KUNIN WE & REED MS (2009) Modelling the coupled dynamics of moorland management and upland vegetation. *Journal of Applied Ecology,* **46,** 278–288.

COX ES (2007) *The implications and effects of bracken (*Pteridium aquilinum *(L.) Kuhn) control on species diversity, re-vegetation and bracken performance*. PhD thesis, University of Liverpool. University of Liverpool, Liverpool.

CRAWLEY MJ (2013) *The R book*, 2nd edn. Wiley, Chichester, UK.

GUREVITCH J & CHESTER JST (1986) Analysis of repeated-measures experiments, *Ecology*, **67**, 255.

HADFIELD JD (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R Package. *Journal of Statistical Software,* **33**, 1–22.

HENDERSON G (1954) Mechanisation of bracken eradication. *Journal of the Institute of British Agricultural Engineers*, 33-44

LE DUC MG, PAKEMAN RJ & MARRS RH (2003) Changes in the rhizome system of bracken subjected to long-term experimental treatment. *Journal of Applied Ecology,* **40**, 508–522.

LEGENDRE P & GALLAGHER ED (2001) Ecologically meaningful transformations for ordination of species data. *Oecologia*, **129**, 271–280.

LEWIS N, GOTHAM P, OTTERBURN B, OVERBURY T, SHEPHERD P & BACON J (1997) Bracken breaking – a bruising battle. *Enact,* **5**, 19-22.

LOWDAY JE, MARRS RH & NEVISON GB (1983) Some of the effects of cutting bracken (*Pteridium aquilinum* (L.) Kuhn) at different times during the summer. *Journal of Environmental Management,* **17**, 373 380.

LOWDAY JE & MARRS RH (1992) Control of bracken and the restoration of heathland. I. Control of bracken. *Journal of Applied Ecology,* **29**, 195–203.

MARRS RH & LOWDAY JE (1992) Control of bracken and the restoration of heathland. II. Regeneration of the heathland community, *Journal of Applied Ecology,* **29**, 204–211.

MARRS RH & WATT AS (2006) Biological flora of the British Isles: *Pteridium aquilinum* (L.) Kuhn. *Journal of Ecology,* **94**, 1272–13.

MARRS RH, FROST AJ, PLANT RA & LUNNIS P (1992) Aerial applications of asulam: a bioassay technique for assessing buffer zones to protect sensitive sites in upland Britain. *Biological Conservation,* **59**, 19-23.

MARRS RH, JOHNSON SW & LE DUC MG (1998) Control of bracken and the restoration of heathland. VIII. The regeneration of the heathland community after 18 years of continued bracken control or 6 years of control followed by recovery, *Journal of Applied Ecology,* **35**, 857-870.

MARRS RH, GALTRESS K, TONG C, BLACKBIRD S J, HEYES TJ, PAKEMAN RJ & LE DUC MG (2007) Conflicts between competing conservation goals, biodiversity or ecosystem services: quantification of element losses and species recruitment using a moorland-bracken model system. *Journal of Environmental Management,* **85**, 1034-1047.

MIATTO RC, SILVA LA, SILVA-MATO, DM, & MARRS RH (2011) Woody vegetation structure of Brazilian Cerrado invaded by *Pteridium arachnoideum* (Kaulf.) Maxon (Dennstaedtiaceae). *Flora,* **206** 757-762.

NAYLOR REL, ed. 2002. *Weed Management Handbook, 9th Edition***.** Wiley-Blackwells, Oxford, UK.

OKSANEN J, BLANCHET FG, KINDT R, LEGENDRE P, MINCHIN PR, O'HARA RB, SIMPSON GL, SOLYMOS P, STEVENS MHH & WAGNER H (2011) vegan: Community Ecology Package, R package version 2.0-2. URL: http://CRAN.R-project.org/package=vegan. [accessed 1/10/2013].

PAKEMAN R J & MARRS R H (1992) The conservation value of bracken *Pteridium aquilinum* (L.) Kuhn-dominated communities in the UK, and an assessment of the ecological impact of bracken expansion or its removal. *Biological Conservation,* **62**, 101–114.

PAKEMAN RJ, LE DUC MG & MARRS RH (2000) Bracken distribution in Great Britain: strategies for its control and the sustainable management of marginal land. *Annals of Botany,* **85B**, 37–46.

PAKEMAN RJ, LE DUC MG MARRS RH, CONDLIFFE I, ENRIGHT D & SMALLSHIRE D (2005) *Bracken control, vegetation restoration and land management.* Rural Development Services Technical Advice Note No. 23. Defra, London. http://www.defra.gov.uk/rds/publications/technical/tan_23.pdf.

PINHEIRO JC & BATES DM (2000) *Mixed effects models in S and S-Plus*. Springer Verlag, New York, USA.

R DEVELOPMENT CORE TEAM (2011) R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/. [accessed 1/10/2013].

RAWES M & WELCH D. (1969) Upland productivity of vegetation and sheep at Moor House National Nature Reserves, Westmorland, England. *Oikos Supplementum,* **11**, 1-69.

ROBINSON RC (1986) Practical herbicide use for bracken control. In: *Bracken: Ecology, Land-use and Control* (ed. RT Smith & JA Taylor), 331–340. Parthenon Press, Carnforth, UK.

ROBINSON RC (2000) The means to large-scale, effective bracken clearance in the UK. In: *Bracken Fern: Toxicity, Biology and Control* (ed. J.A Taylor & R.T Smith), 156–162. International Bracken Group, Aberystwyth, UK

RODWELL JS (1992) *British Plant Communities, Volume 3. Grassland and Montane Communities*. Cambridge University Press, Cambridge.

STACE C (2010) *New Flora of the British Isles, 3rd edn*. CUP, Cambridge, UK.

USEPA (1995) *R.E.D. Facts*: Asulam. United States Environmental Protection Agency. URL: http://www/epa/gov/oppsrrd1/REDs/factsheets/0265fact.pdf. [accessed 1/4/2013].

WILLIAMS GH & FOLEY A (1976) Seasonal variations in the carbohydrate content of bracken. *Botanical Journal of the Linnean Society,* **73**, 87–93.

Supporting information

Additional Supporting Information may be found in the online version of this article.

Figure S1 Experiment Layout of the Bamford Edge Experiment**.**

Figure S2 Bruising in practice: (a) Film of *P. aquilinum* bruising at Bamford Edge; (b-e) various types of bruising equipment.

Figure S3. Photographs of the six control treatments in Block C of the Bamford Edge experiment in 2013, the year after treatments were finished (Photos by W. Chiba).

Figure S4 Application of asulam by various techniques.

Figure S5 Scanning electron microscope photographs illustrating sections of stem from three different bruised fronds showing damage to external and soft tissue layers but intact vascular strands within an endodermis-like structure (abstracted from Cox, 2007).

Figure Legends

Fig. 1. Modelled mean response and raw data of (a) bracken cover (%), (b) frond density (fronds m^{-2}), (c) frond length (cm) and (d) diversity (Shannon-Weiner) to bracken control treatments through time at Bamford Edge, Derbyshire. Treatment codes: Untr = Untreated experimental control, Cutx2 and Cutx3 = cut twice and thrice yearly; Bruisex2 and Bruise x3= Bruise twice and thrice yearly; Asulam = Initial asulam application followed by annual spot spraying of all emergent fronds.

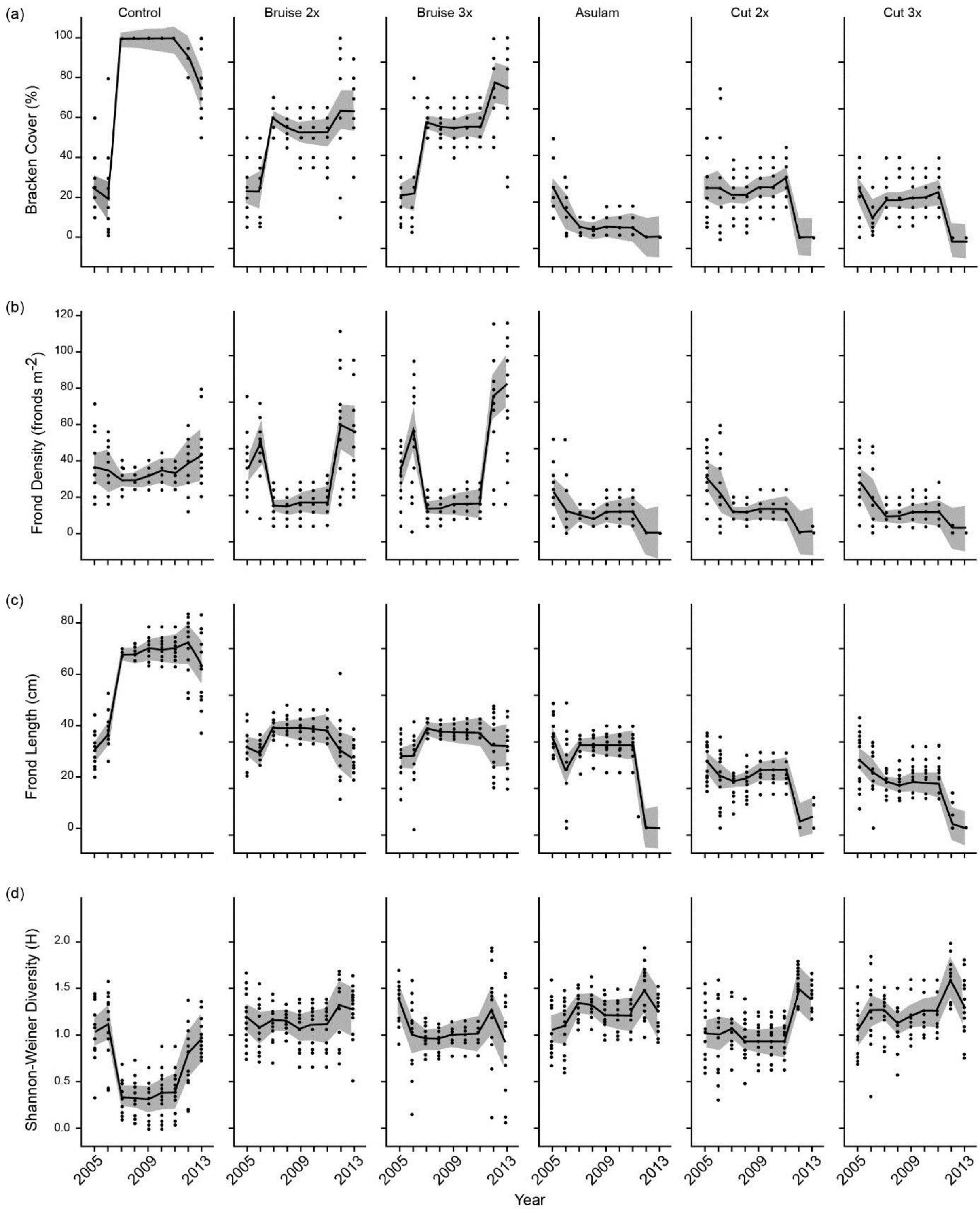
Fig. 2. Change in the herbicide application intensity through time at the whole-plot scale between 2009 and 2012 in the asulam-treatment at Bamford Edge, Derbyshire; intensity was assessed by counting the number of squirts applied per plot during spot-spraying the entire plot (one squirt per frond). Mean values ±SE are presented.

Fig. 3. Frond distribution map of the three most successful treatments in 2013 at the Bamford Edge, Derbyshire; maps of each replicate Block (A, B, C) are presented. Treatment codes: Cutx2 and Cutx3 = cut twice and thrice yearly; Asulam = Initial asulam application followed by annual spot spraying of all emergent fronds.

Fig. 4. Ordination biplots of the first two canonical axes (CC1, CCA2 respectively) derived from the CCA showing: (a) five treatments and their interactions with time (vectors) and (b) species. The origin (0,0) represents the untreated controls in 2005. (a) Treatments: Asulam, Bruise2x is twice-yearly bruising, Bruise3x is thrice-yearly bruising, Cut2x is twice-yearly cutting, and Cut3x is thrice-yearly cutting. (b) Main species group descriptors in each quadrant are denoted in bold; species abbreviations: Ac = *Agrostis capillaris*; Ao = *Anthoxanthum odoratum*; Ap = *Aira praecox*; Av = *Agrostis vinealis*; Cc = *Carex caryophyllea*; Cf = *Cerastium fontanum*; Ci = *Campylopus introflexus*; Cp = *Campylopus pyriformis*; Cpi = *Carex pilulifera*; Cq = Cladonia *squamosa*; Cs = *Carex* spp.; Cv = *Calluna vulgaris*; Df = *Deschampsia flexuosa*; Ds = *Dicranum* scoparium; Ea = *Epilobium angustifolium*; Fe = *Fraxinus excelsior*; Fo = *Festuca ovina*; Fr = *Festuca rubra*; Fu = *Filipendula ulmaria*; Gs = *Galium saxatile*; Hc = *Hypnum cupressiforme*; Hj = *Hypnum jutlandicum*; Hl = *Holcus lanata*; Lb = *Lophocolea bidentata*; Lc = *Luzula campestris*; Lm = *Luzula multiflora*; Ns = *Nardus stricta*; Pa = *Pteridium aquilinum*; Pc = *Ptilidium ciliare*; Pe = *Potentilla erecta*; Pf = *Polytrichum formosum*; Po = *Poa annua*; Pp = *Pseudoscleropodium purum*; Ps = *Pleurozium schreberi*; Ra = *Rumex acetosella*; Rs = *Rhytidiadelphus squarrosus*; Rt = *Rumex acetosa*; Sm = *Stellaria media*; Vm = *Vaccinium myrtillus*; Vo = *Veronica officinalis*. Treatment codes: Untr = Untreated experimental control, Cutx2 and Cutx3 = cut twice

and thrice yearly; Bruisex2 and Bruise x3= Bruise twice and thrice yearly and Asulam = Initial asulam application followed by annual spot spraying of all emergent fronds.

Fig. 5. Scanning electron microscope photographs illustrating sections of stem from three different bruised fronds showing damage to external and soft tissue layers but intact vascular strands within an endodermis-like structure (abstracted from Cox, 2007).



(a)
Control
Bruise 2x
Bruise 3x
Asulam
Cut 2x
Cut 3x
Bracken Cover (%)
(b)
Frond Density (fronds m-2)
(c)
Frond Length (cm)
(d)
Shannon-Weiner Diversity (H)
2005
2009
2013
Year

Fig.1.

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Fig.1.

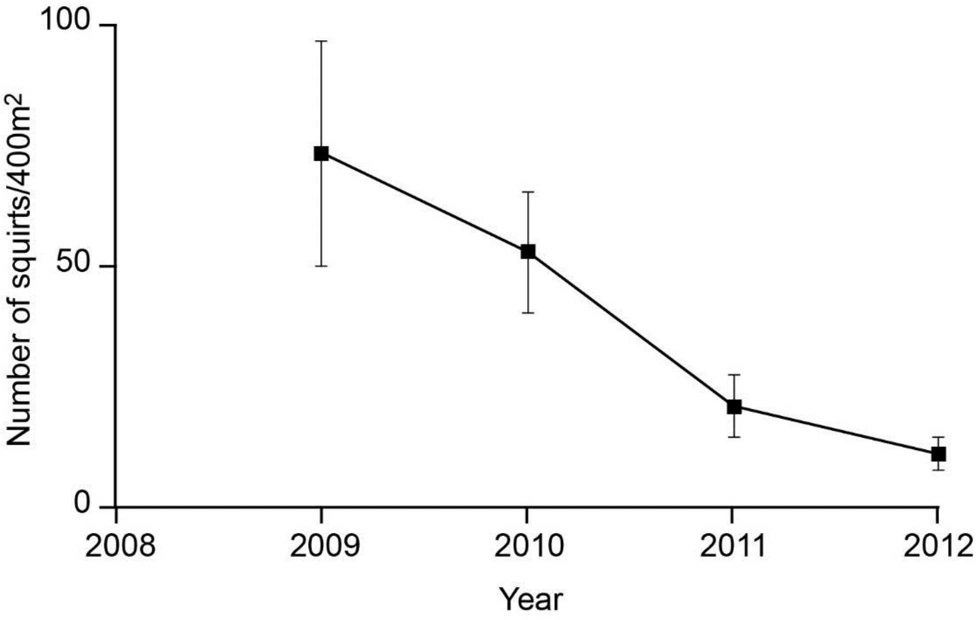
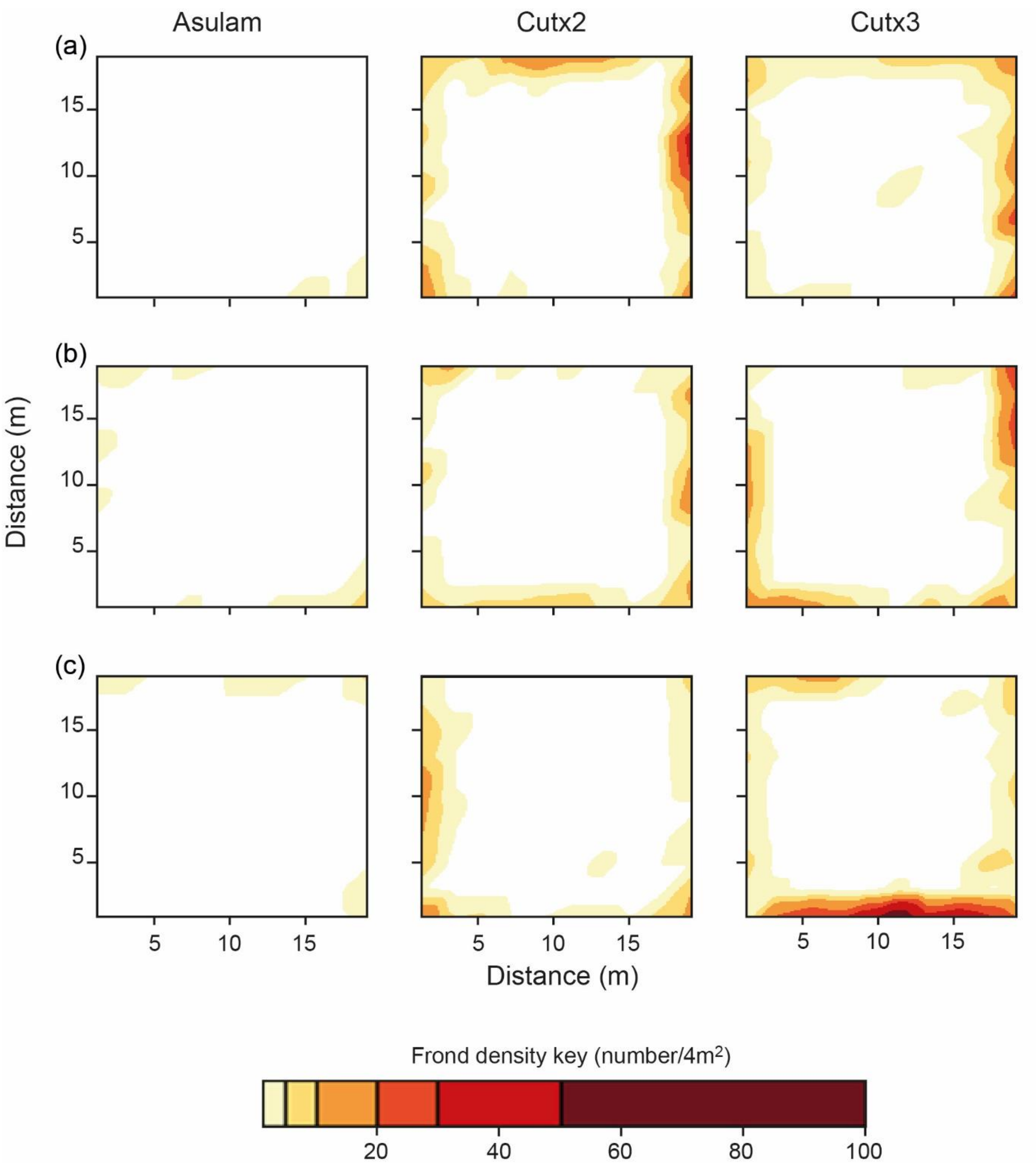

100
50
0
Number of squirts/400m²
2008
2009
2010
2011
2012
Year

Fig. 2

333



Asulam
Cutx2
Cutx3
(a)
(b)
(c)
15
10
5
Distance (m)
5
10
15
Distance (m)
Frond density key (number/4m2)
20
40
60
80
100

Fig. 3

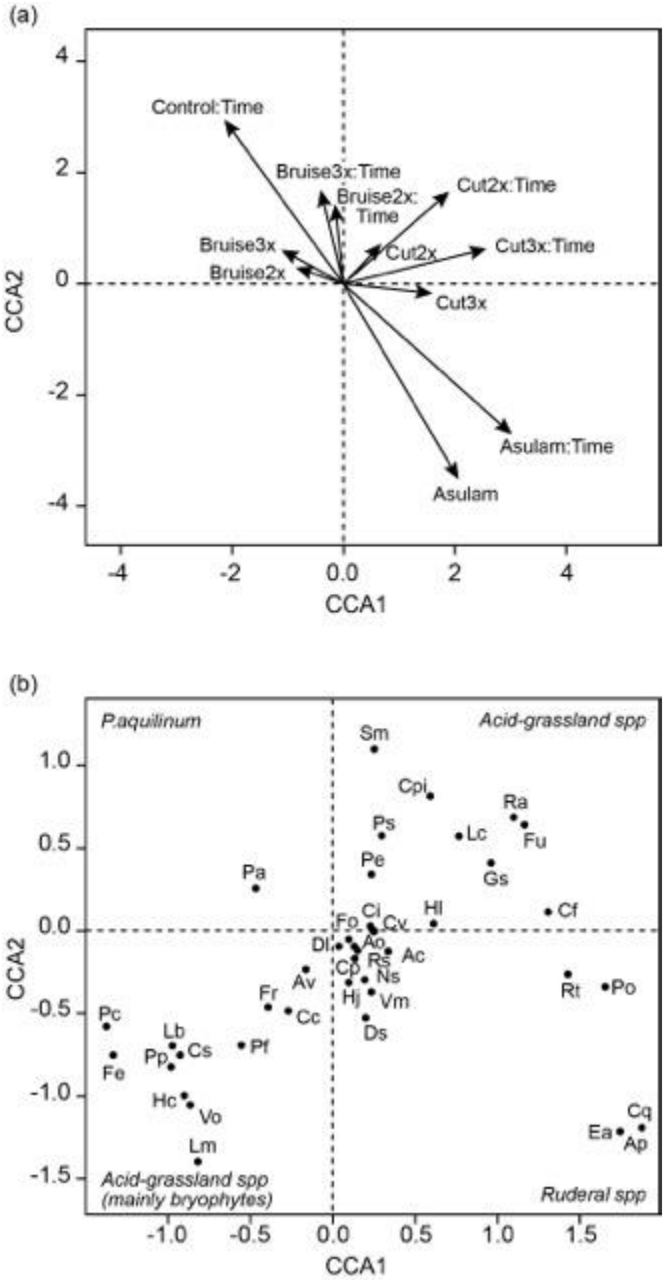
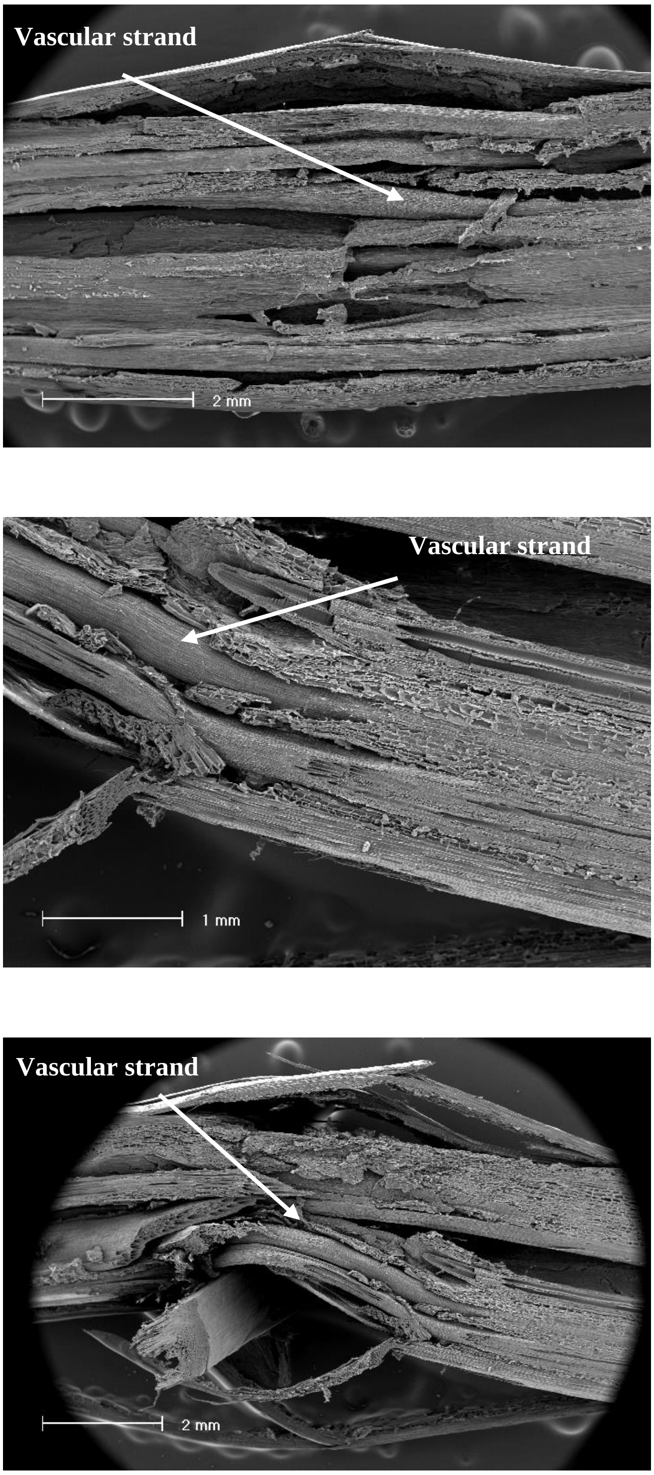

(a)
Control:Time
Bruise3x:Time
Bruise2x:
Time
Cut2x:Time
Bruise3x
Cut2x
Cut3x:Time
Bruise2x
Cut3x
Asulam:Time
Asulam
CCA2
CCA1
(b)
P.aquilinum
Acid-grassland spp
Acid-grassland spp
(mainly bryophytes)
Ruderal spp
Sm
Cpi
Ra
Ps
Lc
Fu
Pe
Gs
Pa
Hl
Cf
Fo
Ci
Cv
Dl
Ao
Ac
Cp
Rs
Ns
Rt
Po
Av
Hj
Vm
Fr
Cc
Ds
Pc
Lb
Cs
Pp
Pf
Fe
Hc
Vo
Cq
Ea
Ap
Lm
CCA2
CCA1

Fig.4

(a)
(b)
(c)



Vascular strand
2 mm
Vascular strand
1 mm
Vascular strand
2 mm

Fig. 5

Table 1. Summary of GLMM posterior mean estimates of the first-order (linear) response of three bracken response variable and Shannon-Weiner index to treatment through time with the lower (L 95%) and upper (U 95%) 95% confidence intervals and associated Bayesian *p*-values (P_{MCMC}). Included are contrasts for each mean estimate with the control obtained using *t*-tests performed on the posterior distributions. Significance of P_{MCMC}: *=P< 0.05, **=P<0.01,**=P<0.001.

Response	Treatment	Effect Through Time					Contrast with Control		
		Mean	L95%	U95%	P_{MCMC}		Difference ± SE	t	P_{MCMC}
P. aquilinum Cover (%)	Untreated	53.38	43.18	63.77	<0.001	***	-	-	-
	Asulam	-17.11	-27.73	-7.32	0.002	**	-70.48 ± 0.14	-957.86	<0.001 ***
	Bruise2x	34.47	24.26	44.80	<0.001	***	-18.90 ± 0.14	-256.09	<0.001 ***
	Bruise3x	50.02	39.85	60.14	<0.001	***	-3.35 ± 0.14	-45.48	<0.001 ***
	Cut2x	-19.30	-28.16	-9.62	<0.001	***	-72.68 ± 0.14	-1029.79	<0.001 ***
	Cut3x	-16.05	-25.11	-7.34	<0.001	***	-69.42 ± 0.14	-1005.91	<0.001 ***
P. aquilinum frond density (No m^{-2})	Untreated	6.30	-6.60	19.58	0.340		-	-	-
	Asulam	-14.88	-28.19	-1.82	0.029	*	-21.18 ± 0.19	-224.23	<0.001 ***
	Bruise2x	15.24	2.38	28.53	0.025	*	8.93 ± 0.18	95.04	<0.001 ***
	Bruise3x	34.70	21.60	48.08	<0.001	***	28.40 ± 0.19	299.85	<0.001 ***
	Cut2x	-23.52	-35.37	-10.59	<0.001	***	-29.83 ± 0.18	-326.46	<0.001 ***
	Cut3x	-19.26	-30.59	-7.70	0.022	*	-25.56 ± 0.17	-288.57	<0.001 ***
P. aquilinum frond length (cm)	Untreated	32.16	-23.95	40.38	<0.001	***	-	-	-
	Asulam	-27.11	-34.80	-18.70	<0.001	***	-59.27 ± 0.12	-1014.34	<0.001 ***
	Bruise2x	-1.97	-10.17	5.81	0.621		-34.13 ± 0.11	-585.61	<0.001 ***
	Bruise3x	3.77	-4.31	11.87	0.338		-28.40 ± 0.11	-488.15	<0.001 ***
	Cut2x	-16.07	-22.76	-8.94	<0.001	***	-48.23 ± 0.11	-884.87	<0.001 ***
	Cut3x	-21.98	-28.42	-15.41	<0.001	***	-54.15 ± 0.11	-1019.07	<0.001 ***
Shannon-Weiner Index	Untreated	-0.16	-0.42	0.10	0.230		-	-	-
	Asulam	0.20	-0.07	0.46	0.131		0.36 ± 0.00	188.69	<0.001 ***
	Bruise2x	0.12	-0.15	0.38	0.365		0.27 ± 0.00	144.67	<0.001 ***
	Bruise3x	-0.12	-0.39	0.14	0.345		0.03 ± 0.00	17.23	<0.001 ***
	Cut2x	0.34	0.10	0.57	0.009	*	0.49 ± 0.00	275.09	<0.001 ***
	Cut3x	0.31	0.09	0.52	0.006	*	0.46 ± 0.00	268.21	<0.001 ***

Supporting Information

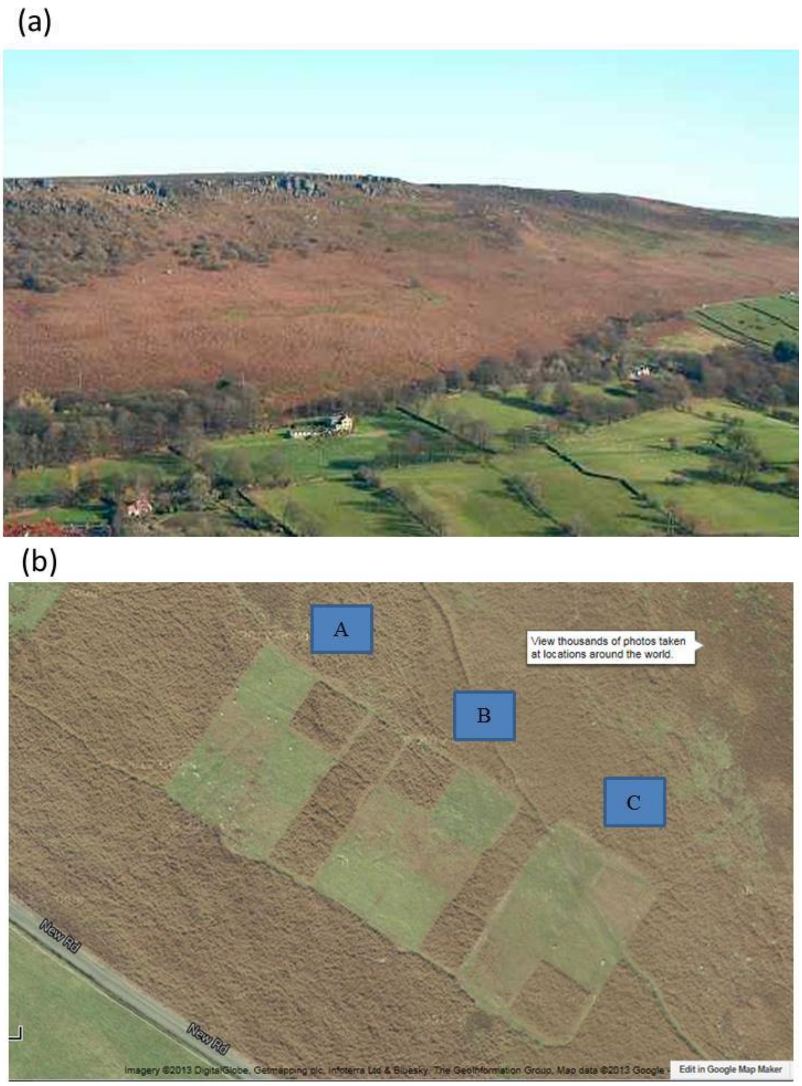
Fig. S1 Experiment Layout of the Bamford Edge Experiment**.**

The experiment at Bamford Edge (SK 213 841) was set up in November 2004. *P. aquilinum* control treatments were applied for the first time at this site, in the summer of 2005. The treatments were:

(a) Cut twice per year
(b) Cut thrice per year
(c) Bruised twice per year
(d) Bruised thrice per year
(e) Sprayed with asulam in 2005 then spot sprayed in following years
(f) Untreated "experimental control"

Blocks are located 20m apart, from north-west to south-east (left to right of the picture) in the order A, B, C. All treatments applied randomly within Blocks.

Treatments were applied between 2005-2012.



View of Bamford Edge experimental area (a) before treatment and (b) after treatment note differential density of sheep on the treated plots.

Experimental design: three blocks with treatment plots randomly allocated; each treatment plot= 20m x 20m separated by 2m strips,

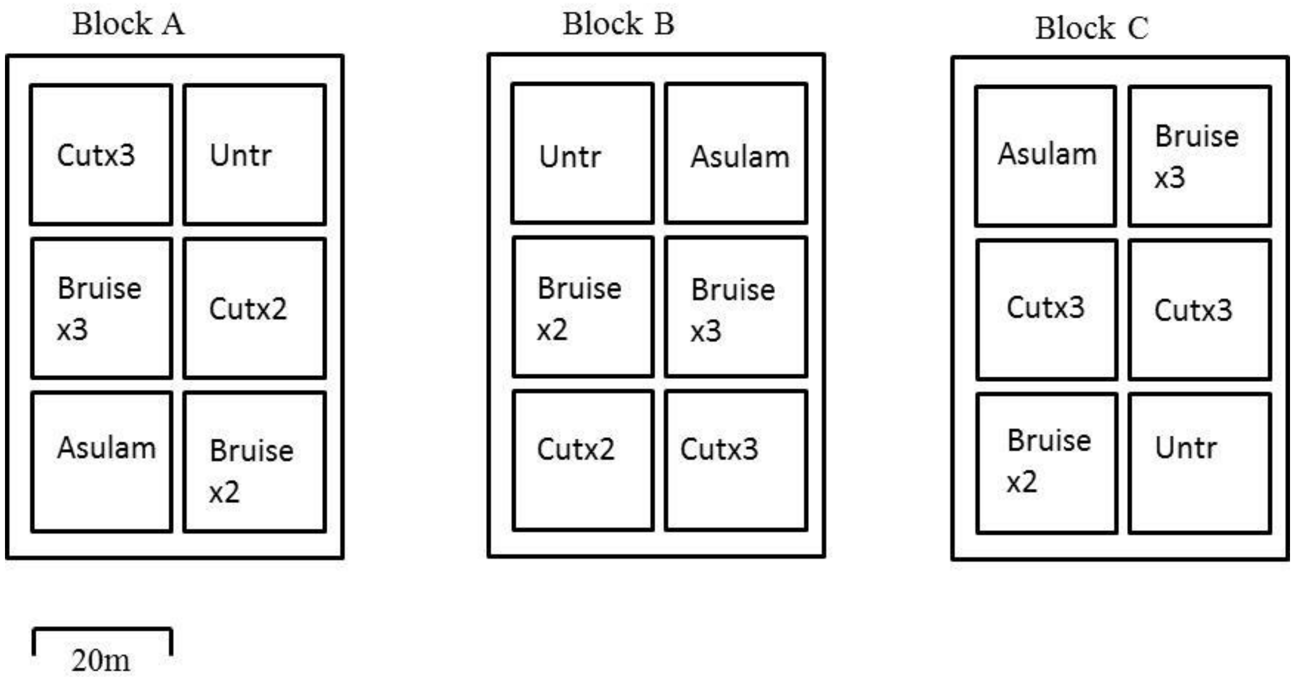


Fig. S2 Bruising in practice: (a) Film of *P. aquilinum* bruising at Bamford Edge; (b-e) photographs of various types of bruising equipment.

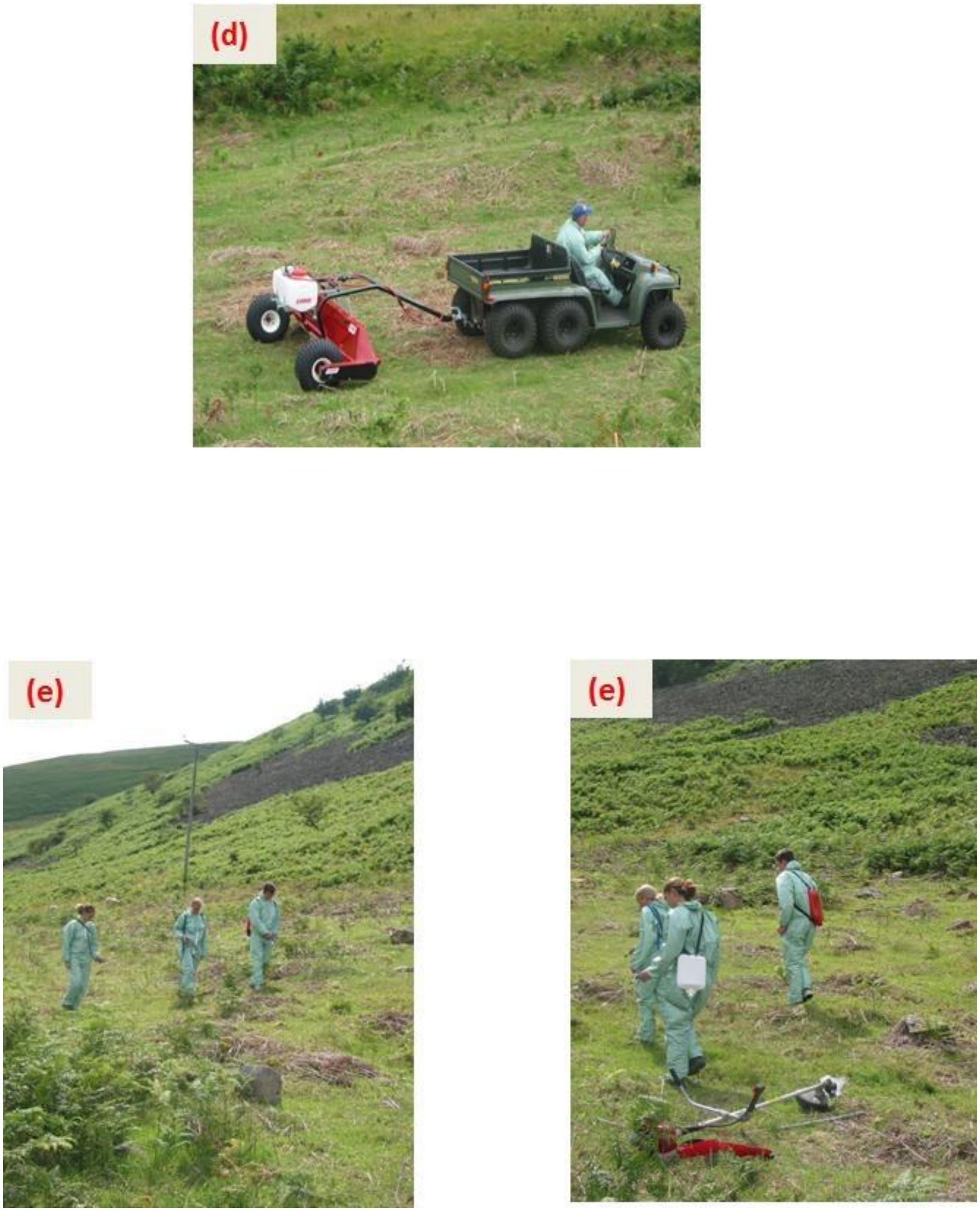
(a) http://www.youtube.com/watch?v=s6gb8sK4FgI&feature=youtu.be.
(b-d) Simple trailed bruiser (Bruisers by Peter Gotham Ltd) showing three units towed in gang formation; these can be turned through 180 and towed easily on the road, (d) shows them in use in relatively-dense *P. aquilinum*.
(e) Bruiser being pulled by heavy-horse (Timber-Tinker and "Sally")

Fig. S3 Photographs of the six control treatments in Block C of the Bamford Edge experiment in 2013, the year after treatments were finished (Photos by W. Chiba).

http://www.youtube.com/watch?v=s6gb8sK4FgI&feature=youtu.be

Full film uploaded when accepted.

Fig. S4 Application of asulam by various techniques. (a) asulam from the air; (b) spot-spraying apparatus illustrating ATV-mounted sprayer with 4 distribution lances (equipment from Landward Consultancy, Malton, UK); (c) spot-spraying in action using (b) and driver plus four lance operatives – the four hand-held lances cover a swathe of ca. 40 m; (d) spot-application using a weed-wiper (example shown is by Logic, Hexham, UK) – here herbicide is only applied to the tallest emergent fronds, and (e) using hand-held applicators – the AccuDos 2/25P spray applicators (manufactured Micron Sprayers Ltd, Bromyard, Hereford, UK **–** these are small backpacks containing herbicide solution and the operative squirts a 1-2 ml dose of herbicide on to each frond; it is helpful to have at least 4 operatives on the ground to give fast cover over large areas. Most of the technology for spot-spraying has been developed by Dr R. Robinson (now Landward Consultancy, Malton, UK).


(d)
(e)
(e)